The Sporogony and Systematic Position of the Aggregatidæ.

By

Helen L. M. Pixell-Goodrich, B.Sc.,
Beit Memorial Research Fellow.

With Plate 13.

The very noticeable parasites infesting the alimentary canal of many Cephalopods were recorded by Eberth (**3**) so long ago as 1862. The genus comprising these forms has suffered many changes of nomenclature. In 1875 it was named Benedenia by Schneider (**21**, Note xiii, p. xl) to include B. octopiana from the octopus, and this generic name was employed so late as 1900 by Schaudinn (**20**, p. 203). In Schneider's second paper (**22**) in 1883 the parasite was referred to throughout as Klossia instead of Benedenia, as the author stated that he was convinced that the two genera could not be distinguished. In this Siedlecki followed him in his two papers of 1898 (**23** and **24**) on the parasites of Sepia, which he considered to be identical with those of the octopus, although he found only three or four sporozoites in a sporocyst, whereas Schneider had described eight to fifteen in Klossia octopiana, and Labbé had already given the specific name of K. eberthi (**7**) to the species inhabiting Sepia. In a footnote to his second paper, however, Siedlecki (**24**, p. 800) stated that after seeing Laveran's work on K. helicina he thought the original name of Benedenia should be readopted. It was then discovered that this name had in 1858 been appropriated for a Trematode genus, therefore Blanchard, in 1900, altered it to Légeria. Jacquement (**5**)

in 1903 pointed out that this had been already applied to a polycystid Gregarine, and again changed the name to Légerina. This, however, was never used; for, in the previous year, after Siedlecki had stated that, like the so-called Eugregarines, these parasites did not appear to undergo Schizogony, the genus had been called Eucoccidium by Lühe (**11**). By this generic name the parasites were generally known until, in 1906, the excellent researches of Leger and Duboscq (**8** and **9**) showed that the schizogony, which had been supposed to be absent from the life-history of the genus, took place in the Crab, Portunus, where Frenzel (**4**) had described it in 1885 as a Gregarine under the name of Aggregata; this name is therefore now universally applied to the genus which I hope to prove is undoubtedly Coccidian in character as assumed by Siedlecki. Moroff (**15**) in 1906 claimed to have shown that the fertilisation was of the gregarine type; that is, that the macrogametocyte gave rise before fertilisation to a number of small cells, and that these were the true macrogametes, each of which gave, after fertilisation, a single spore. In 1908, in a later work of great length, Moroff (**16**) admitted that he might have been mistaken in his description of the process of fertilisation, and no longer insisted on these parasites being Gregarines, stating that further investigations were necessary. It was for this reason that, at Prof. Minchin's suggestion, I undertook this study of Aggregata, which has extended over some years. During this time I have examined Cephalopods, chiefly Sepia, from Plymouth, the Solent, and more recently from the Mediterranean in the neighbourhood of Banyuls-sur-Mer. To the authorities and staff, especially to the Director, Prof. Pruvot, of the Laboratoire Arago, I should like to express my thanks for their hospitality and assistance; also to Prof. Minchin, not only for suggesting such an interesting subject for investigation, but for much kind help given me during the years that I have been privileged to work in his Department at the Lister Institute. Since last October the work has been carried on in Oxford, and I am much indebted to

Prof. G. C. Bourne for the kind interest he has shown in the progress of the investigations, and for allowing me the use of a laboratory in his department. My husband, Mr. E. S. Goodrich, has also given me much advice and assistance.

As pointed out above, Aggregata octopiana of the octopus and A. eberthi of Sepia have long been known, but in addition to these, Moroff (**16**, p. 144) claims to have distinguished some eight or nine more species in Octopus and five in Sepia. The distinguishing characteristics given, being chiefly those of size, are not at all conclusive, and, so far as my observations go, I have been unable to corroborate their existence.

According to Léger and Duboscq (**10**) A. eberthi can undergo its schizogony in Portunus arcuatus and P. holsatus as well as in P. depurator (**10**, p. 90 et seq.), but not in P. puber. L. Their attempts to infect other Crustacea, such as Inachus dorsettensis, Carcinus maenas, etc. (**10**, p. 95), failed, although some of these, for instance, Inachus dorsettensis, are sometimes found in nature infected with a species of Aggregata (Smith, **19**). Possibly the natural cephalopod host of the latter is an octopus; it does not, at any rate, seem to be Sepia officinalis. Many experiments such as these appear to me to be necessary before we can hope conclusively to distinguish different species of Aggregata, the possible existence of several of which I am not prepared to deny.

Material and Technique.

It is very essential to have the material quite fresh, and in this connection a method of killing Cephalopods so as to eliminate the difficulty of dealing with the emission of ink may be of interest. This is to slip a loop of fine, though strong string round the head just above the mantle, and suddenly to pull the ends very tightly. In this way the animal is immediately strangled—the general paralysis being too quick to allow time for any ink to be thrown out, and at the same time quantities of blood are left in the heart and may be drawn off to be used as a mounting medium if required.

All Sepia officinalis, whether from Plymouth, the Solent or Banyuls, have been found to be infected : but none of the numerous specimens of Sepia elegans, which occurred in quantities near Banyuls-sur-Mer, had any infection during September, neither had the Eledone and Loligo that were examined.

Both Sepia officinalis and Eledone from Banyuls were almost universally infected with the Cestode Scolex polymorphus, the occurrence of which has already been recorded by Monticelli (**14**) from Naples. In Sepia officinalis the region of the alimentary canal most infected was the spiral stomach between the entrance into the muscular stomach and the longitudinal ridges which run up into the rectum. The latter chiefly contained large cysts, which, being somewhat over one millimetre in diameter, were quite visible to the naked eye. For examination in the fresh state some of the tissue from the spiral stomach was teased out, in sea-water as a general rule, though sometimes intestinal fluid or blood were found to be preferable. Films and portions of the spiral stomach were fixed in Schaudinn's fluid, ordinary corrosive sublimate and acetic acid, or mixtures of the latter with equal quantities of 4 per cent. formalin—this last perhaps giving on the whole the best results. Iron hæmatoxylin was by far the most satisfactory nuclear stain. Ehrlich's and Delafield's hæmatoxylin mixtures were also used, as well as paracarmine, borax carmine and many cytoplasmic stains, such as Lichtgrün and picric acid, eosin or orange G.

Vegetative Stages.

Since Léger and Duboscq (**10**), Moroff (**16**), and others have already given excellent figures of merozoites, trophozoites, and sporozoites, we can begin our study with the adult trophozoite ripe for reproduction. At this stage there is certainly a delicate limiting membrane present—a fact denied by Siedlecki (**24**, p. 804),—also a large vesicular nucleus, containing, as a rule, a single large karyosome. In some

cases the cytoplasm contains large irregular masses, which stain intensely with iron-hæmatoxylin and other chromatin stains. These would seem to be metabolic products of the cytoplasm, such as Comes (1) has described in Stenophora, rather than of nuclear origin, and to bear no obvious relation to the processes of reproduction described below. Smaller particles of more uniform size, probably of similar nature, are usually scattered throughout the cytoplasm. The ripe trophozoites vary enormously in size, the longer diameter of some reaching 400 μ, while others may be only about 50 μ; and, since all intermediate dimensions can be found, it does not seem possible to distinguish several species, either in this or subsequent stages, by size alone. Therefore I cannot support Moroff's conclusions on this point (**16**, pp. 146 and 147).

Formation of the Gametes.

While the distinction between males and females becomes very pronounced later on, it is extremely difficult to trace the differences back to the trophozoite stage. However, individuals which are to become females appear, on the whole, less dense, and have their cytoplasm more vacuolated than those destined to be males. The females also as a rule grow to a larger size. After a considerable resting period at the completion of growth the early stages leading up to the formation of the gametes are gone through very rapidly.

In the female there is, first, a migration of the nucleus towards the surface, from which, however, it remains separated by a plug of dense, finely granular protoplasm, which gives off radiations, spreading in all directions through the cytoplasm (fig. 1). The central part of this protoplasmic plug rises up on the surface, forming a cone of attraction, and, after entrance of the microgamete, appears to sink in again, carrying the male chromatin with it (figs. 2 and 3).

In the male the nucleus also approaches the surface and rapidly divides by a peculiar method of mitosis. A centrosome with distinct astral radiations apparently emerges from

the nucleus at the beginning of the process, and undergoes repeated division before the nucleus becomes subdivided. These centrosomes, with their conspicuous asters, spread over the surface of the microgametocyte, giving rise to such polymitotic figures as those given by Moroff (**16**, fig. 50, pl. vi). In the meantime the nucleus becomes irregularly lobed, the lobes growing out in various directions towards the asters. The peripheral lobes eventually become constricted off as separate nuclei, into which the greater part of the chromatin has migrated. At a later stage the nuclei, containing deeply staining chromatic networks, are found evenly distributed over the whole surface. Throughout this process the centrosomes and asters take up a position between the nuclei and the surface of the gametocyte. The peripheral nuclei continue to undergo repeated binary division, preceded in every case by the division of the centrosome and aster. As the nuclei become more numerous the surface at their disposal is increased by the formation of rounded lobes, which gradually become nipped off as separate spheres, covered with developing microgametes (fig. 13). A fully developed male generally consists of several such spheres, sometimes as many as six or seven. The nuclear divisions described above are very similar to those which take place in the formation of the sporoblasts in the zygote, and this fact may have contributed in some measure to Moroff's unfortunate mistake in thinking that these sporoblasts represented the macrogametes and were separately fertilised. It is not clear which process some of his figures are intended to represent; but his figs. 53 and 55 (**16**, pl. vii) appear to me to be stages in the formation of microgametes. The differences to be observed between them will be again referred to (p. 168) after the description of the formation of the sporocytes.

Siedlecki described the nuclear division of the male as taking place by a process of chromidial fragmentation (**24**, p. 815), such as has been described in other Coccidia. This error may, I think, be partly ascribed to the fact that he worked chiefly with films, which he stated (**24**, p. 801) that

he found to be better than sections. I, on the contrary, have found that, owing to the large size and great density, especially of the male gamete, it is practically impossible to make out the very rapid nuclear changes which take place at the beginning of the process without studying an extensive series of sections.

The chromatin of the developing microgametes becomes more and more concentrated until the nucleus is reduced to a small, densely staining mass. This, surrounded by a certain amount of cytoplasm, projects on the surface of the microgametocyte and becomes elongated, carrying the centrosome at its distal extremity. The microgamete now becomes more and more elongated, the nucleus lengthens out, and the stalk connecting it with the residual sphere of cytoplasm becomes drawn out to form the tail region. Two flagella make their appearance at the free end, and the microgamete gradually assumes its fully developed form.

The mature microgametes during life measure about 40 μ in length, which is very much the same as the fixed ones (fig. 11). These lengths do not, of course, include the flagella, which are generally considerably more than half the length of the body of the microgamete. The latter is strap-shaped, with a more or less cork-screw nucleus running through the anterior half. This, at any rate, appears to be the form that the nucleus takes during the phase at which it emerges from the microgametocyst.

It may be of interest to recall that a spiral nucleus has also been recorded in Trypanosoma brucei (Robertson **18**, p. 236) and in the so-called male forms of T. lewisi described and figured by Prowazek (**17**, figs. 35 and 36). In some cases I have observed a faint wavy line extending along the tail, but, except for this, there is no sign of an undulating membrane. In front of the nucleus and at the base of the flagella are situated the granules, which I take to be blepharoplasts. In deeply stained specimens they frequently appear as one large granule. These basal granules are apparently derived from the original centrosome of the

younger stage. During this time the limiting membrane surrounding the male gametocyte becomes considerably thicker. The male individual therefore becomes enclosed in a distinct cyst, which is, however, thinner than that in the female. At an early stage the gametocyte shrinks away from the cyst-wall, becoming separated from it by fluid in which it is suspended, while the microgametes develop on its surface (fig. 13). The cyst is sufficiently resistant to be dissected out of the host tissue, and the microgametes, after the development of their flagella, have been observed actively swimming about inside. Upon the rupture of this cyst they swarm out, but I did not succeed in making them retain their activity for any length of time, although many different media were employed for mounting them, including Sepia, blood and intestinal fluid, as well as ordinary sea-water and saline of varying densities. On this account it was not easy to make out the details of their movement, but flagella could be seen being vigorously lashed in front, and, in addition, the flattened, somewhat expanded tail was used in propulsion.

Fertilisation.

In the fresh state numerous microgametes have been observed crowded round a macrogamete, but only one appears to enter. This it does by the cone of attraction, and on reaching and penetrating the female nucleus its chromatin rounds itself off into granules, as shown in figs. 2 and 3. Moroff's figures (**16**, pl. xi, fig. 91, and text-figs. M_1 and M_2) doubtless represent this stage, although he failed to discover the true meaning, as he was convinced that fertilisation was of the Gregarine type. The macrogamete nucleus has assumed an oval or pear-shaped form by the time the microgamete enters. A network of spindle threads appears running irregularly along the long axis of the nucleus from the point of entrance of the microgamete to the opposite pole. Meanwhile the karyosome undergoes changes. The chromatic substance emerges from the micropyle as a stream of spherules which arrange themselves along the spindle

threads. In some cases strings of granules can be seen inside the karyosome and coming from it; possibly the spindle threads themselves arise in this way. Somewhat similar irregular spindles have been described in other Coccidia; for example, in Coccidium proprium by Siedlecki (**25**) and in Orcheobius herpobdellæ by Kunze (**6**), and no doubt the nuclear fibres figured by Moroff (**16**, pl. iii, fig. 16, and text-fig. N. 2) are of the same nature.

The granules of male chromatin which have become lodged at the superficial end of the nucleus stream inwards, and presumably mingle with the granules of female chromatin on the spindle threads: fertilisation is thus accomplished.

Directly after the entrance of the microgamete a resistant cyst appears round the zygote, which thereupon contracts and comes to lie freely suspended in fluid. A similar contraction has been described as taking place in Coccidium proprium (**25**), but in this species the oocyst is formed quite early, and a micropyle is left for the entrance of the microgamete.

Sporogony.

After fertilisation, upon the break-up of the spindle, the nucleus of the zygote becomes reconstituted, and this synkaryon undergoes mitosis. A centrosome and aster make their appearance on the outer side of the nucleus: they divide, and their daughter-centrosomes and asters separate, accompanied by lobes of the synkaryon, into which passes the chromatin, arranged on spindle fibres in the form of irregular loops (fig. 10). The first few nuclear divisions follow one another in such quick succession that, before the chromatin threads of one spindle have completely separated, the centrosomes have again divided, and the next pair of spindles are being formed approximately at right angles to the preceding one, as shown in fig. 10, where the large asters so generally to be seen are also figured. In this way an extensively branching nucleus is formed, the central region only disappearing at a later stage.

This peculiar form of mitosis in the fertilised female may be considered as intermediate between ordinary mitosis and the polymitosis described above in the male. In the latter the nucleus lags so far behind the centrosomes in its division, and so disturbs the sequence of events, that the connection between the division of the two organs is obscured or lost. Moroff (**16**) has already given numerous beautiful figures of these stages.

After repeated division of the centrosomes and asters large daughter-nuclei with numerous small faintly staining granules are constructed off near the surface (fig. 12). The stage with about twenty to fifty of these is a commonly occurring one, and would seem to indicate a somewhat prolonged resting period. It can generally only be distinguished from the corresponding resting stage in the formation of the microgametes by the larger size and lesser affinity for chromatin stains exhibited by its nuclei. The large early sporoblast nuclei contain, as a rule, small evenly distributed granules of chromatin only, whereas the small early male nuclei have large deeply staining masses, or threads, of chromatin.

This resemblance between the early stages of division in the microgametocyte and those in the zygote is of considerable interest, since it seems to support the view that the two processes correspond to one another, and that not only is the macrogamete of the female homologous with the microgametocyte of the male, but also that the microgamete of the male corresponds to the sporoblast of the female.

Further, whereas in Gregarines the adult female gives rise to small cells (macrogametes) which become separately fertilised and then form the zygotes (sporoblasts), in the Coccidia the adult female gives rise to the small cells (sporoblasts) after fertilisation. Thus the chief difference between the processes of sporogony in the two groups is that fertilisation takes place at an earlier stage in Coccidia than in Gregarines. This could be shown in tabular form as follows, the arrow representing fertilisation :

Coccidia.

♀ trophozoite → macrogamete × n sporoblasts → n sporocysts × mn sporozoites.

♂ trophozoite → microgametocyte × n microgametes.

Gregarines.

♀ trophozoite → macrogametocyte × n macrogametes → n sporocysts × mn sporozoites.

♂ trophozoite → microgametocyte × n microgametes.

Cuénot, in 1901 (**2**, p. 632), remarked that fertilisation was tardy in Gregarines and precocious in Coccidia, but refused to commit himself to a homology of the various steps. The evidence I have given above is, however, in agreement with the theory put forward by Minchin (**12**, pp. 271–274) in 1903 as to the homologies that exist between the gametes of Coccidia and those of Gregarines, namely, that the sporoblasts of Coccidia (formed by the division of the zygote after fertilisation) "may be compared to those of Gregarines by supposing that the process of multiplication, by which the gametocyte of the Coccidia gave rise primitively to a number of female gametes, has not been completely suppressed, but merely deferred until after the process of zygosis" (**12**, p. 272). The same theory is again referred to in 1912 (**13**, p. 354).

During the resting stage in sporoblast formation, as in the same stage of the male, very distinct centrosomes and asters are generally apparent, being situated on pointed processes projecting from the surface. The next stage is marked by the repeated division of these centrosomes and asters, followed by the division of the nuclei. During this time the whole body of the zygote becomes more or less folded, thus increasing the area of the surface on which the nuclei are situated, and giving room for their increasing number. This process corresponds to the breaking up of the microgametocyte into separate spheres at the stage when its nuclei are rapidly increasing in number.

One very usual method of folding in the dividing zygote is for one pole to become invaginated, as in a gastrula; this was noticed by Schneider (**22**, pl. ix, fig. 24). It is clear that when this cup-like body is cut in one direction an arc of cytoplasm surrounded by nuclei will result such as described by Moroff as characteristic of his A. arcuata (**16**, pp. 118 and 146), just as, when cut through a plane at right angles to the former direction, a ring may result.

The effect of this folding and increase of nuclei is to diminish the relative amount of cytoplasm compared to nuclei, so that when the sporoblasts round off there is never any great quantity of residuum, and in most cases none at all. At this stage, therefore, the oocyst is generally crowded with sporoblasts. In these the centrosome and aster persist for some time, forming a projection on the surface (fig. 4), but I have not been able to trace their division preceding nuclear division to form the sporozoites. Before this happens the whole projection sinks down and the nucleus assumes a resting form (fig. 5). On resuming its activity the nucleus divides into two, but though a spindle is formed, no asters are visible. During this first division the sporocyst makes its appearance (fig. 6). In the vast majority of cases only one of these secondary nuclei divides again (fig. 7), and in this way are formed the nuclei of the three sporozoites (figs. 8 and 9) which are normally present. Only occasionally do both of the secondary nuclei divide again, forming four sporozoites. Each sporozoite has at its posterior end an elongated deeply staining nucleus in which no karyosome can be distinguished (fig. 9).

Sometimes streams of sporocysts are to be observed passing along blood spaces, although, in the Sepia, no tendency can be seen on their part to open in order that auto-infection may take place. The whole oocyst is generally said to pass into the lumen of the Cephalopod's alimentary canal whence it reaches the exterior. So far no definite evidence of the escape of unbroken oocysts has been obtained, and many of them are very deeply embedded in the thick connective tissue

of the wall far from the lining epithelium. Possibly it is by eating the cysts in dead Cephalopods that crabs generally become infected. About the process of schizogony in the crab nothing further need be said, for it has been beautifully described by Leger and Duboscq (**10**).

Systematic Position.

In having this alternation of hosts corresponding to its alternation of generations, the genus Aggregata differs from all other Coccidia whose life-histories are at present known, and on this account would constitute a distinct family, the Aggregatidæ making a third family of Section A of the Eucoccidia in the classifications drawn up by Minchin (**13**, p. 352). The family could not easily find a place in the classification given by Leger (**10**a, p. 86), except that it would belong to the Eimeridea, which corresponds to Minchin's Section A. The subdivisions of Leger's two sections are based on the number of sporozoites in the oocyst, which Minchin (**13**, p. 353) has suggested is not a natural method of classification. This suggestion is supported by the fact that in the Aggregatidæ, now to be included in Coccidia, there is no constancy in the number of sporozoites, A. eberthi having only three or four in each of its numerous sporocysts, while A. octopiana, according to Moroff (**16**), has sixteen, and other species have from eight to twenty-four.

Summary.

(1) The genus Aggregata is undoubtedly to be included among the Coccidia—the fertilisation being not Gregarine in character as described by Moroff (**15** and **16**), but typically Coccidian. In Aggregata eberthi, here described, from Sepia officinalis, the large female gamete is fertilised by the small, active, biflagellate microgamete, and the zygote so formed gives rise to a large number of sporoblasts.

(2) The polymitotic nuclear divisions giving rise to the microgametes are so similar to those giving rise to the sporoblasts that they afford some evidence in favour of the view that these stages are homologous. It is remarkable also that the microgametes further resemble the sporoblasts in being enclosed in a distinct cyst.

References.

1. Comes, S.—"Untersuchungen über den Chromidialapparat der Gregarinen," 'Archiv. Protistenkunde,' x, 1907, pp. 416–439.
2. Cuénot, L.—"Recherches sur l'évolution et la conjugaïson des Gregarines," 'Archiv. de Biol.,' xvii, 1901, pp. 581-652.
3. Eberth, C. J.—"Über die Psorospermienschläuche der Cephalopoden," 'Zeitschr. wiss. Zool.,' Bd. xi, 1862, pp. 397–399.
4. Frenzel, J.—"Übereinige in seetieren lebende Gregarinen," 'Arch. Mikr. Anat.,' Bd. xxiv, 1885, pp. 545–588.
5. Jacquement, M.—"Sur la systématique des Coccidies des Cephalopodes," 'Archiv. Protistenkunde,' ii, 1903.
6. Kunze, W.—"Orcheobius herpobdellæ," 'Archiv. Protistenkunde,' ix, 1907, p. 382.
7. Labbé, Alphonse.—"Recherches Zoologiques, cytologiques et biologiques sur les Coccidies," 'Arch. Zool. expér.' (3), iv, 1896, pp. 515–654.
8. Léger, L., and Duboscq, O.—"Sur l'évolution des Gregarines gymnosporées des Crustacées," 'C. R. Acad. Sc. Paris,' vol. cxlii, 1906, pp. 1225–1226.
9. ———"L'évolution d'une Aggregata de la Seiche chez le Portunus depurator," 'C. R. Sc. Soc. de Biol.,' vol. lx, 1906, pp. 1001–1003.
10. ———"L'évolution schizogonique de l'Aggregata (Eucoccidium) eberthi (Labbé)," 'Archiv. Protistenkunde,' xii, 1908, pp. 44–108.

10a. Léger, L.—"Caryospora simplex . . . et la classification des Coccidies," 'Archiv. Protistenkunde,' xxii, 1911, pp. 71–89.

11. Lühe, M.—"Über Geltung und Bedeutung der Gattungsnamen Eimeria und Coccidium," 'Centralbl. f. Bakt. v. Parasit.,' Abt. xxxi, 1902.
12. Minchin, E. A.—'The Sporozoa: a Treatise on Zoology' (Lankester). London: A. & C. Black, 1903.
13. ———'An Introduction to the Study of the Protozoa.' London: Edward Arnold, 1912.

14. Monticelli, F. S.—"Fauna elmintologica Golfe Napoli," 'Mittl. Stat. Zool. Neapel.,' viii, 1888, pp. 85–152.

15. Moroff, Theodor.—"Sur l'évolution des prétendues Coccidies des Céphalopodes," 'C. R. Ac. Sc. Paris,' vol. cxlii, 1906, pp. 652–654.

16. ———"Die bei den Cephalopoden vorkommenden Aggregataarten als Grundlage einer kritischen Studie über die Physiologie des Zellkernes," 'Archiv. Protistenkunde,' xi, 1908, pp. 1–224.

17. Prowazek, S.—"Studien über Säugetier trypanosomen, I," 'Arb. Kais. Gesundheitsamte Berlin,' xxii, 1905, pp. 351–395.

18. Robertson, Muriel.—"Notes on certain Blood-inhabiting Protozoa," 'Proc. R. Phys. Soc. Edin.,' xvi, 1906, pp. 232–247.

19. Smith, Geoffrey.—"Note on a Gregarine (Aggregata inachi n. sp.), etc.," 'Mitt. Zool. Stat. Neapel,' xvii, 1905, pp. 406–410.

20. Schaudinn, F.—"Untersuchungen über den Generations-wechsel bei Cocciden," 'Zool. Jahrb. abt. Anat.,' Bd. xiii, 1900, pp. 197–292.

21. Schneider, Aimé.—"Note sur la Psorospermie oviforme du poulpe," 'Arch. Zool. expér.,' vol. iv, Note xiii, 1875, pp. xl–xlv.

22. ———"Nouvelles observations sur la Sporulation du Klossia octopiana," 'Arch. Zool. expér.,' 1883, pp. 77–104.

23. Siedlecki, M.—"Reproduction sexuée . . . de la Coccidie de la Seiche (Klossia octopiana)," 'C. R. Soc. Biol. Paris' (10), v, 1, 1898, pp. 540–543.

24. ———"Étude cytologique . . . de la Coccidie de la Seiche," 'Ann. Inst. Pasteur,' vol. xii, 1898, pp. 799–836.

25. ———"Reproduction sexuée . . . chez Coccidium proprium," 'C. R. Soc. Biol. Paris' (10), v, 1898, pp. 664–666.

EXPLANATION OF PLATE 13,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on "The Sporogony and Systematic Position of the Aggregatidæ."

[Preparations stained iron-hæmatoxylin and drawn with a 2 mm. lens and compensating ocular 12, giving approximately a magnification of 2000, unless otherwise stated.]

Fig. 1.—Part of a section through a macrogamete ready for fertilisation, with cone of attraction (*co.*) and radiating protoplasmic plug (*pp.*)